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REVIEWS

The Origin and Fate of Liver Myofibroblasts

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SUMMARY

We describe potential phenotypes of hepatic stellate cells and portal fibroblasts activated in response to toxic and cholestatic liver injury and outlined the possible mechanisms of their activation, and pathways of their targeting for antifibrotic therapy.

Liver fibrosis of different etiologies is a serious health problem worldwide. There is no effective therapy available for liver fibrosis except the removal of the underlying cause of injury or liver transplantation. Development of liver fibrosis is caused by fibrogenic myofibroblasts that are not present in the normal liver, but rather activate from liver resident mesenchymal cells in response to chronic toxic or cholestatic injury. Many studies indicate that liver fibrosis is reversible when the causative agent is removed. Regression of liver fibrosis is associated with the disappearance of activated myofibroblasts and resorption of the fibrous scar. In this review, we discuss the results of genetic tracing and cell fate mapping of hepatic stellate cells and portal fibroblasts, their specific characteristics, and potential phenotypes. We summarize research progress in the understanding of the molecular mechanisms underlying the development and reversibility of liver fibrosis, including activation, apoptosis, and inactivation of myofibroblasts. ***(Cell Mol Gastroenterol Hepatol 2024;17:93–106; https://doi.org/10.1016/j.jcmgh.2023.09.008)***

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Introduction

Hepatic fibrosis develops in response to chronic hepatotoxic or cholestatic liver injury and is characterized by damage of hepatocytes, development of inflammatory responses, activation of collagen type I–producing myofibroblasts, accumulation of extracellular matrix (ECM) proteins, and formation of fibrous scar, which makes liver fibrotic and cirrhotic.[1,2]

Hepatotoxic liver injury can be caused by viral hepatitis B virus (HBV) or hepatitis C virus (HCV), excessive alcohol consumption (alcohol-associated liver disease [AALD]), and nonalcoholic fatty liver disease (NAFLD). Chronic HBV and HCV infections were the main cause of liver fibrosis during the past 2 decades.[3] The introduction of effective therapies for HBV and HCV has decreased these viruses and etiological agents of liver fibrosis. However, NAFLD is on a rise, and the development of metabolic syndrome and insulin resistance is becoming a major risk factor of liver fibrosis in obese patients.[4–7] NAFLD progresses from steatosis (nonalcoholic fatty liver) to nonalcoholic steatohepatitis (NASH).[8,9] It is estimated that NAFLD affects around 1 billion people worldwide, which may account for about 25% of the global population.[10] A total of 20% of patients with nonalcoholic fatty liver may progress to NASH and develop steatohepatitis, fibrosis, and cirrhosis.[9,11] Excessive alcohol consumption can further exacerbate liver injury in NASH patients. Development of AALD often occurs in patients with metabolic syndrome who have a body mass index >25–27 kg/m^2.[12]

NASH-induced liver fibrosis is characterized by pericellular and perisinusoidal fibrosis.[9,13] The pathogenesis of NASH has been described by a "two-hit" theory.[14] The first hit involves metabolic injury of hepatocytes, hepatocyte

Abbreviations used in this paper: **AALD, alcohol-associated liver disease; α-SMA, α-smooth muscle actin; aHSC, activated hepatic stellate cell; aPF, activated portal fibroblast; BDL, bile duct ligation; BM, bone marrow; CCl_4, carbon tetrachloride; CTGF, connective tissue growth factor; E, embryonic day; ECM, extracellular matrix; eHSC, embryonic hepatic stellate cell; ER, endoplasmic reticulum; GFAP, glial fibrillar acidic protein; HBV, hepatitis B virus; HCV, hepatitis C virus; HGF, hepatic growth factor; HSC, hepatic stellate cell; iHSC, inactivated hepatic stellate cell; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; IL, interleukin; INF, inflammatory; LSEC, liver sinusoidal endothelial cell; MMP, matrix metalloproteinase; MSLN, mesothelin; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cholangitis; PDGF, platelet-derived growth factor; PDGFRβ, platelet-derived growth factor receptor β; peHSC, persistent embryonic hepatic stellate cell; PF, portal fibroblast; PMSC, portal mesenchymal stem cell; PSC, primary sclerosing cholangitis; qHSC, quiescent hepatic stellate cell; rqHSC, recovery-associated quiescent hepatic stellate cell; SAMe, scar-associated mesenchymal cell; SASPs, senescence-associated secreted proteins; scRNA, single-cell RNA; SLIT2, slit guidance ligand 2; snRNA, single-nucleus RNA; TGF-β, transforming growth factor β; VIM, vimentin.**

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damage, development of endoplasmic reticulum stress, reactive oxygen species production, and excessive lipid accumulation.[2,15] The second hit is caused by activation of inflammatory responses, recruitment of bone marrow (BM)–derived neutrophils and myeloid cells, activation of liver resident Kupffer cells, and secretion of inflammatory (interleukin [IL]-6, tumor necrosis factor α, IL-1β, IL-17, IL-13, and others) and fibrogenic cytokines (transforming growth factor β1 [TGF-β1], connective tissue growth factor [CTGF], platelet-derived growth factor [PDGF], leptin).[2] Inflammatory and fibrogenic cytokines facilitate de novo lipogenesis in hepatocytes,[16] alter the cross-talk between hepatocytes, liver sinusoidal endothelial cells (LSECs) and hepatic stellate cells (HSCs),[17,18] leading to the loss of LSEC fenestration,[19] HSC activation into fibrogenic myofibroblasts,[20] progression of liver fibrosis, and decline of the detoxifying liver function.[2,8,21–23]

Cholestatic fibrosis leads to activation of hepatic myofibroblasts and development of liver fibrosis.[24,25] Cholestatic fibrosis results from the impaired bile production or bile passage due to malfunction of bile secretion by hepatocytes, or mechanical obstruction in the bile ducts.[26] Cholestatic liver diseases include primary sclerosing cholangitis (PSC), primary biliary cholangitis (PBC), and secondary biliary obstruction (triggered by obstruction of the bile ducts by liver malignancies or gall bladder stones).[27] PSC (an autoimmune disease caused by destruction of the small intrahepatic bile ducts) and PBC (idiopathic disease that causes damage to the intrahepatic and/or extrahepatic bile ducts) are the most prevalent cholestatic liver diseases.[28] The incidence of both PSC and PBC is on the rise,[29] ranging from 0 to 16.2 per 100,000 people for PSC and 1.9 to 40.2 per 100,000 people for PBC.[30] Pediatric patients' cholestatic fibrosis is often caused by biliary atresia, a genetic disorder associated with the occlusion of intrahepatic/extrahepatic bile ducts in the liver, and is often linked to other genetic abnormalities.[8] Increased levels of toxic bile acids in the liver and plasma cause injury and apoptosis of hepatocytes and cholangiocytes, leading to the proliferation of the bile ducts (ductular reaction), massive inflammation, and activation of portal fibroblasts (PFs) and HSCs that produce collagen type I in the course of cholestatic fibrosis.[27,31–33]

Despite the differences in etiology, activation of myofibroblasts is a common mechanism in the pathogenesis of liver fibrosis.[34,35] Hepatic myofibroblasts exhibit stellate or spindle shape, upregulate the expression of the cytoskeletal proteins such as α-smooth muscle actin (α-SMA), vimentin (VIM), and nonmuscle myosin.[2,35] Extensive studies have demonstrated that liver resident HSCs and PFs are the major sources of hepatic myofibroblasts. BM-derived fibrocytes are also implicated as a minor contributor to the myofibroblast population.[2] Meanwhile, genetic cell fate mapping of epithelial cells produced no evidence that hepatocytes, cholangiocytes, or their progeny contribute to the population of myofibroblasts through epithelial mesenchymal transition in experimental models of liver fibrosis.[36,37]

Here we will discuss the potential sources of hepatic myofibroblasts, and summarize the findings of the rapidly evolving single-cell technologies, which have broadened our understanding of the myofibroblast diversity and heterogeneity, and were instrumental in identification of new targets for antifibrotic therapy.[38] Hence, HSCs will be the major focus of the current review, due to their immense contribution to liver fibrosis of different etiologies.

Origins of Myofibroblasts in Liver Fibrosis

Potential Sources of Myofibroblasts in the Fibrotic Liver

Myofibroblasts are not present in the normal liver but activate in response to chronic liver injury. TGF-β is the key factor that drives activation of hepatic myofibroblasts.[39] Several other cytokines have also been implicated in myofibroblast activation and differentiation, such as CTGF + IL-13, and PDGF, and to lesser extent IL-6, leptin, and IL-17.[2,8] The sources of hepatic myofibroblasts have been extensively discussed in the literature. It is believed that liver resident mesenchymal cells, activated hepatic stellate cells (aHSCs), and activated PFs (aPFs) are the major contributors to myofibroblasts in fibrotic liver (Figure 1). Other populations, such as liver mesothelial cells, liver capsule progenitors, and BM-derived fibrocytes and/or circulating mesenchymal stem cells, were also implicated in the pathogenesis of liver fibrosis, but their role in liver fibrogenesis is much less understood.

Genetic labeling of hepatic myofibroblasts in healthy and fibrotic livers

Genetic Labeling of Hepatic Myofibroblasts Revealed the Key Role of HSCs in the Pathogenesis of Toxic Liver Fibrosis. Development of transgenic collagen-α1(I)-GFP (Col-GFP) mice, in which all collagen type I–expressing cells upregulate GFP in real time,[40,41] made it possible to monitor activation status of liver resident mesenchymal cells in healthy or chronically injured livers. These mice are a useful tool to study hepatic myofibroblast biology, gene expression profiles, and mechanism of activation. Under physiological conditions, hepatic mesenchymal cells do not express collagen type I. Studies of Col-GFP mice revealed that only a few GFP^+ cells were found around central vein in the livers of 2-month-old mice.[42] Those GFP^+ cells that did upregulate collagen type I lacked characteristics of myofibroblasts and did not secrete ECM.[40] GFP^+ myofibroblasts arise in response to chronic liver injury as a result of activation/differentiation of liver resident mesenchymal cells. Over 95% of fibrogenic myofibroblasts comprised aHSCs and aPFs in experimental models of hepatotoxic and cholestatic fibrosis in mice.[42,43] HSCs were identified by expression of desmin and neural markers glial fibrillar acidic protein (GFAP),[8] nerve growth factor receptor,[44,45] and vitamin A.[46–50] Expression of thymocyte differentiation antigen 1 (Thy1) 1,[51–53] fibulin 2,[51] elastin,[54] mesothelin (Msln), mucin16 (Muc16), asporin (Aspn), basonuclin 1 (Bnc1), uroplakin-β (Upk1β), calcitonin-related polypeptide α (Calca), and glipican 3 (Gpc3) and lack of vitamin A expression distinguished Col-GFP^+VitaminA^- aPFs from

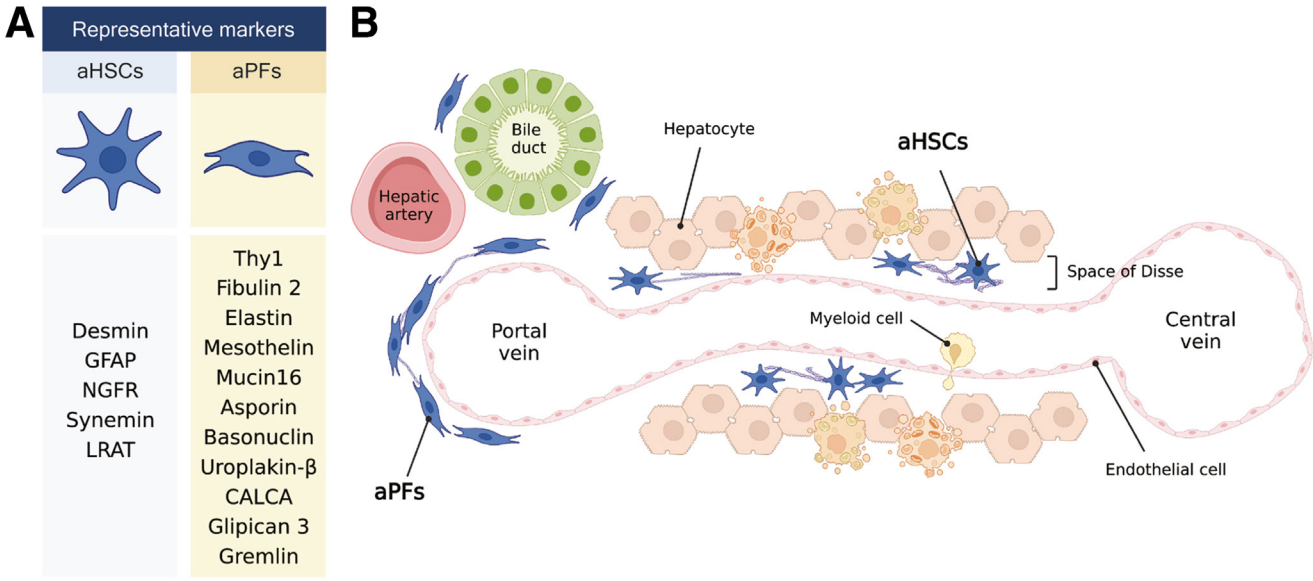

Figure 1. Contribution of HSCs and PFs to hepatic myofibroblasts. (*A*) Representative markers that can differentiate aHSCs from aPFs. (*B*) HSCs are located in the space of Disse (between sinusoidal endothelial cells and hepatocytes) and store vitamin A. PFs are located around portal triads, and under physiological conditions maintain the integrity of the portal tract. There are no myofibroblasts in the normal liver. In response to chronic liver injury HSCs rapidly activate and give rise to collagen type I–producing myofibroblasts. PFs activate into myofibroblasts mainly in response to cholestatic injury.

Col-GFP$^+$VitaminA$^+$Desmin$^+$GFAP$^+$ aHSCs (Figure 1*A*).[42,55] We[41] and others[56] have demonstrated that HSCs gave rise to the majority (>85%) of myofibroblasts in response to carbon tetrachloride (CCl_4) and intragastric ethanol. aPFs and fibrocytes contributed 8% and 4% of total myofibroblasts, respectively, but only moderately upregulated collagen type I,[50,57,58] indicating that aHSCs are the major source of myofibroblasts in response to toxic liver injury.

In normal liver, HSCs contribute ~10% of the total number of liver cells, which roughly correspond to one-third of the nonparenchymal cell population.[42,59] In response to chronic (2 months) CCl_4-induced liver injury in mice, the population of aHSCs expands up to 16%–17% of total liver cells, but upon CCl_4 cessation (1 month), the number of HSCs is gradually decreased to ~6% of total liver cells.[41] The HSC population was largely restored to normal levels (~10%) after 6 months of CCl_4 cessation,[41] suggesting that HSC repopulation of livers recovering from liver fibrosis is critical for maintaining normal liver function.

Genetic Labeling Revealed That aPFs Trigger the Development of Cholestatic Fibrosis. In normal liver, PFs comprise a small population of "periductular mesenchymal cells" (~0.1% of liver cells) that maintain integrity of the portal tract.[27,60,61] Under physiological conditions, PFs do not secrete ECM and do not produce α-SMA. Studies of bile duct ligation (BDL)–injured collagen-GFP mice have revealed that aPFs are the first responders to cholestatic injury caused by surgical obstruction of the common bile duct (BDL) in mice. Similar results were obtained using another model of cholestatic injury, Mdr2$^{-/-}$ mice (deficient of canalicular phospholipid flippase, Mdr2/Abcb4, and develop disruption of bile duct tight junctions and basal membranes, bile leakage, and periportal cholestatic fibrosis),[62] that resembles PSC[63–68] and mimics MDR2 deficiency in patients.[66,68,69] At the onset of cholestatic injury (5 days post-BDL), aPFs comprised ~70% of liver myofibroblasts and strongly upregulated collagen type I (to the level similar to that observed in CCl_4-activated HSCs).[55] While aHSCs contributed to only 20% of myofibroblasts at the onset of BDL (5 days), progression of cholestatic fibrosis was characterized by rapid activation of aHSCs into myofibroblasts (~55% at 14 days of BDL) and at 21 days of BDL aPFs comprised 75% of total myofibroblasts.[55] Similar to CCl_4, fibrocytes contributed to 3%–4% of collagen type I–expressing cells in BDL-injured mice.[42] Studies of BDL-injured collagen-GFP mice also suggested that some mesothelial cells residing in the Glisson's capsule can facilitate liver fibrogenesis.[70,71]

Genetic Cell Fate Mapping of Hepatic Myofibroblasts in Fibrotic Livers

Cell Fate Mapping Revealed HSC Phenotypes. The Cre-loxP–based[72] cell fate mapping became a gold-standard method to trace myofibroblast populations and their progeny to determine their activation/differentiation under physiological conditions or in response to injury.[56] Crossing of Collagen-α1(I)^{Cre} mice with Rosa26$^{floxP\text{-}Stop\text{-}floxP\text{-}YFP}$ mice resulted in generation of Col1a1^{YFP} mice.[41] Upon induction of toxic liver injury, all myofibroblasts and their progeny were irreversibly labeled by YPF expression in CCl_4-treated Col1a1^{YFP} mice.[41] Histological and flow cytometry-based analysis revealed that >85% of YFP$^+$ myofibroblasts were comprised of activated HSCs (aHSCs). Cessation of CCl_4 administration resulted in rapid (within 3–6 days) apoptosis of ~50% of activated aHSCs/myofibroblasts, while the other ~50% of aHSCs/myofibroblasts gradually

underwent inactivation and obtained "quiescent-like" phenotype.[73,74] Inactivation of HSCs was associated with downregulation of fibrogenic genes,[56] regression of liver fibrosis, and resolution of fibrous scar (1 month after CCl_4 cessation). Similar results were obtained using $Col1a2^{YFP}$ mice, generated by crossing of Collagen-$\alpha2(I)^{Cre}$ mice with $Rosa26^{floxP\text{-}Stop\text{-}floxP\text{-}YFP}$ mice.[41] The cell fate mapping approach also identified several nonconventional HSC phenotypes, including HSCs activated during embryonic development (embryonic HSCs [eHSCs], >80% of total HSCs at embryonic day 21 [E21]), 20% of which persisted in adult 2-month-old mice (persistent eHSCs [peHSCs]) but were progressively replaced by the newly produced quiescent HSCs (qHSCs).[41] In addition, the cell fate mapping of HSCs in recovering livers revealed that YFP-negative HSCs without history of collagen type I expression emerged in the mouse livers (starting after 2 weeks of CCl_4 cessation),[41] suggesting that HSCs possess unique plasticity, and the replenishment of HSC population(s) might be critical for the maintenance of normal liver function. Hence, the HSC progenitor source still remains to be identified.

With the development of single-cell RNA (scRNA) sequencing, less abundant HSC phenotypes, such as inflammatory and proliferative, were described and linked to HSC activation.[75,76] Although their function and contribution to liver fibrosis and/or liver homeostasis is not well understood, characterization of these newly discovered populations may provide an invaluable insight to distinct functional properties of HSC populations. Much less is known about the fate of aPFs.

Cell Fate Mapping of aPFs. The lineage tracing of aPFs is limited by the availability of the PF-specific Cre-expressing mice. Lrat-Cre–based cell fate mapping of hepatic myofibroblasts (using Lrat-Cre transgenice mice that express Cre throughout embryonic development to the adulthood) confirmed that HSCs were the major contributors to liver fibrosis of different etiologies but did not discriminate between differences in the aPF numbers in response to cholestatic vs toxic liver fibrosis.[43] Because Lrat is not expressed by aPFs in adult mice,[42,43] genetic labeling of aPFs with Lrat-Cre reporter transgene results from transient expression of Lrat-Cre in mesenchymal/mesothelial progenitors during embryonic development. In support, liver mesothelial cells uniquely expressed GPM6A (glycoprotein M6A), PDPN (podoplanin), and WT1 (Wilms tumor 1).[70] During liver development, mesothelial cells migrated inward from the liver surface and gave rise to both HSCs and PFs.[77] Generation of inducible Msln-ER-Cre mice became instrumental in labeling aPFs, tracing their progeny in response to cholestatic liver injury.[71] Morphologically, the expression of Msln-ER-Cre transgene was observed throughout the liver (but not in liver capsule) in BDL-injured mice and was enriched in the portal areas.[55] Studies of $Wt1^{CreERT2}$x $Rosa26mTmG^{flox}$ ($R26TG^{fl/fl}$) mice revealed that in response to injury, hepatic VIM^+ capsular fibroblasts, $GPM6A^+$ and $PDPN^+$ mesothelial cells proliferated from the Glisson's capsule and contribute to subcapsular liver fibrosis but did not significantly migrate into the liver parenchyma.[77]

Undoubtedly, HSCs play a critical role in the pathogenesis of NASH, AALD, and cholestatic fibrosis (Figure 1*B*). The discovery of HSC-specific surface markers, intracellular cytoskeletal proteins, and secreted ECM proteins played a key role in the identification of the distinct HSC phenotypes and revealed significant plasticity and heterogeneity of HSCs and their sublocalization within the lobular structure in normal and diseased livers.[78] Initially, only 2 functional HSC phenotypes were described: qHSCs and aHSCs.[78] In response to chronic toxic injury, qHSCs activated into collagen type I–expressing myofibroblasts,[8] and made the liver fibrotic. Historically aHSCs became the primary targets for antifibrotic therapy.[8,34,79,80]

Hepatic Stellate Cells

In 1876, HSCs were first described by Dr Kupffer using electron microscopy, and characterized them as liver cells enriched in vitamin A droplets.[81] Dr Ito rediscovered HSCs that were located in the space of Disse (between hepatocytes and sinusoidal endothelial cells), and called them hepatic lipocytes.[82,83] HSCs play an important role in wound healing and liver repair.[39] Historically, focal HSC activation was designed to constrain infection and/or encapsulate necrotic area with collagen fibers. HSCs facilitate liver regeneration.[84] Transient HSC activation was also observed in livers subjected to partial hepatectomy in mice.[85] In turn, systemic activation of HSCs into fibrogenic myofibroblasts occurred only in response to persistent chronic liver injury and dysregulation of the physiological repair program, leading to hepatocyte apoptosis and production of inflammatory and fibrogenic chemokines/cytokines.[2]

Major HSC Phenotypes.

Quiescent HSCs. Under physiological conditions, qHSCs reside in the space of Disse, express neural markers (GFAP, synemin, synaptophysin, nerve growth factor receptor 1)[44,45] and lipogenic genes (PPARγ, Adipor1, Adpf, C/EBPd, and others), and store vitamin A.[82] qHSCs can be distinguished from other cell types in the liver by accumulation of vitamin A–containing lipid droplets. HSCs store up to 70%−95% of all Vitamin A/retinol in the body.[86] Formation of lipid droplets in qHSCs is dependent on the enzymatic activity of Lrat.[87] Expression of Lrat is associated with quiescent HSC phenotype and serves as a critical marker of qHSCs. Transcriptional regulation of qHSCs is mediated by PPARγ (regulates expression of lipogenic genes in qHSCs),[88] ETS1/2, GATA4/6 and IRF1/2 transcription factors (identified as HSC lineage-determining transcription factors),[89] and other transcription factors which maintain quiescent HSC phenotype. Deletion of PPARγ, ETS1, GATA4/6 specifically in HSCs, was shown to accelerate HSC activation, and exacerbate the development of liver fibrosis in mice (Figure 2).[82,89,90]

qHSCs mediate multiple functions in the liver, including maintenance of the hepatocyte/LSEC/HSCs niche and homeostasis in the space of Disse. They provide intercellular communications, secretion of local cytokines (IL-6, ET1) and chemokines (CCL2, CCL5), regulate vascular tone and sinusoidal blood flow, and promote hepatocyte proliferation and liver regeneration.[91] qHSCs trigger the synthesis of polypeptide mediators, erythropoietin, and components of the

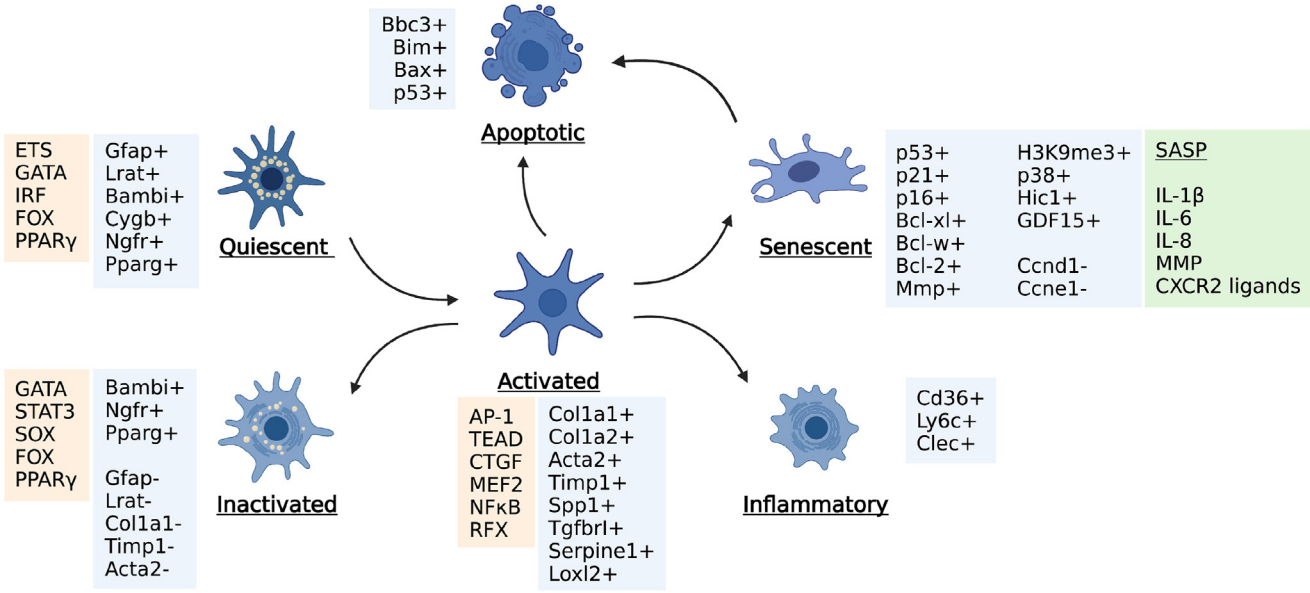

Figure 2. Characterization of phenotypic changes in HSCs in response to chronic injury and fibrosis recovery. Under physiological conditions, HSCs exhibit a quiescent state (qHSCs). In response to chronic liver injury, release of TGF-β1, qHSCs activate into collagen type I–expressing aHSCs. Upon removal of the underlying etiological cause of injury, liver fibrosis can regress, and aHSCs can senesce and apoptose, or inactivate. Inactivation of HSCs (iHSCs) downregulates expression of fibrogenic genes and upregulates expression of some but not all quiescence-associated genes. Phenotype-specific expression of the signature genes (blue boxes) and transcription factors (yellow boxes), and senescence-associated secretory phenotype (SASP) (green box) are shown.

plasminogen activation system that maintain homeostasis in the microenvironment of the hepatic sinusoid.[92] qHSCs are often referred as fat-storing liver pericytes that maintain the integrity of the hepatic lobular structure due to the synthesis of various basal membrane matrix components, such as laminin and nonfibrogenic collagen type IV.[92,93] Recent studies have suggested that qHSCs also provide structural support for hepatic parenchymal cells and serve as a liver scaffold. qHSCs were also implicated in the mediation of immune tolerance (Figure 2).[94]

Quiescent to weakly activated HSCs were enriched in genes and pathways related to cytokines and growth factors. Hepatic growth factor (HGF) was highly expressed in the cytokine-expressing HSCs. HGF was predominantly found in HSCs, while its receptor MET was primarily expressed in hepatocytes in both mice and human liver scRNA sequencing and single-nucleus RNA (snRNA) sequencing datasets. Deletion of Hgf in HSCs through Lrat-Cre in mice produced approximately 70% reduction of Hgf expression levels in normal and fibrotic livers. Cytokine-expressing HSC-enriched HGF protects hepatocytes against death and reduces HCC development. Hgf deletion in HSCs increased HCC development by injection with DEN and CCl_4, and hepatocyte injury in the dietary HF-CDAA high-fat NASH mouse model.[95]

Activated HSCs. In response to chronic liver injury and release of TGF-β, qHSCs rapidly downregulate expression of vitamin A and retinol- and lipid-associated enzymes (such as Lrat, Adipor, Adpf, and others), neural markers (GFAP, synemin, synaptophysin, nestin), and quiescence-associated nuclear receptors (RXR, FXR, LXR, PXR, and PPARγ), and activate into aHSCs/myofibroblasts that upregulate expression of α-SMA, Col1a1, Col1a2, TGFβRI, TIMP1, Spp1, VIM, LoxL1, Spp1, PDGF receptor β (PDGFRβ), and other fibrogenic proteins; secrete ECM proteins, mainly collagen type I; and make the liver fibrotic.[2,8] Moreover, aHSCs migrate from the space of Disse to the fibrotic septa (Figure 2).[34,55,80,96,97] Differentiation of HSCs into myofibroblasts is driven by activation of JunB and AP-1 transcription factors. TEAD, MEF2, Runx1, and Smad2/3 were shown to regulate different aspects of aHSC activation, including suppression of quiescence-associated transcription factors (ETS, GATA, IRF, and others).[2,89,92] Increased reactive oxygen species production induces endoplasmic reticulum stress in the damaged liver, causing release of damage-associated molecular patterns that further accelerate HSC activation. Gut permeability is increased in NASH, and gut-derived lipopolysaccharide–Toll-like receptor 4 signaling promotes production of inflammatory cytokines, growth factors, and HSC activation.[92] In addition, Toll-like receptor 4 signaling can indirectly activate HSCs by decreasing the expression of the TGF-β decoy receptor BAMBI.[92,98] Activated HSCs upregulate TGF-β, IL-6, IL1β, monocyte chemoattractant protein-1/CCL2, and intercellular adhesion molecule 1.[92]

Several cytokines can directly induce the transcription of collagen type I in HSCs. The most potent stimulator is TGF-β, which drives activation of HSCs via SMAD2- or SMAD3-dependent signaling pathway.[99] Activation of SMAD2 or SMAD3 promotes transcription of collagen type I, whereas SMAD7 functions as a suppressor of this transcription in an auto-regulatory feedback loop.[78] CTGF, IL-13, and IL-17

were implicated in facilitation of Col1a1 transcription in the TGF-β–independent manner.[100] IL-17 was also shown to directly induce collagen type I via activation of the Stat3 signaling pathway.[101] Proliferation of aHSCs is regulated by PDGFRβ, while CCL and CCL2 play a critical role in aHSC motility and migration.[92]

Senescent HSCs. In the course of injury, some aHSCs may undergo senescence, a condition characterized by accumulation of nonproliferating cells that secrete pro-inflammatory cytokines and chemokines.[102,103] HSC senescence is triggered by activation of p21 (CDKN1A, which is downstream of phospho-p53 and acts as an inhibitor of the cell cycle by blocking progression through G1/S when associated with CDK2)[104] and p16 (INK4A, that plays an important role in the response to DNA damage signals caused by telomere dysfunction) (Figure 2).[74]

Development of the HSC senescence-associated phenotype is caused by the cell cycle arrest, chromatin instability, DNA damage, mitogenic stress, mitochondrial damage, and secretion of senescence-associated secreted proteins (SASPs).[74,105,106] SASPs serve as an important molecular signature for senescence. SASPs of HSCs include proinflammatory cytokines, such as IL-1β, IL-6, and IL-8; chemokines, such as CXCL-2[107]; and proteases, such as matrix metalloproteinases (MMPs).[74] These factors induce inflammation and attract immune cells to remove senescent cells.[108] Senescent HSCs have been observed in cirrhotic livers of human patients, with the cells expressing p21 and p16 colocalized with those expressing α-SMA. The upregulation of SASP markers was confirmed in cultured primary human activated HSCs that were triggered to senesce by treatment with a DNA-damaging agent.[74] SASPs in senescent HSCs not only promote cancer progression,[109] but also promote tumor clearance by the immune system and inhibit tumorigenesis in the liver.[110]

Cellular senescence is regulated on an epigenetic level via changes in histone modification enzyme H3K9me3 (trimethylation of lysine 9 of histone H3) that causes chromatin repression and subsequent cell cycle silencing. E2F genes and Hic1 are among the targets of H3K9me3.[111,112] Hic1 suppression which is regulated at the epigenetic level in senescent cells was linked to impaired DNA repair and cell cycle suppression.[113] Remarkably, reduced replicative potential of senescent HSCs[74] is accompanied by increased survival due to upregulation of Bcl-XL, Bcl-2, BCL-W, Bcl2L2, and BIM genes and activation of the multiligand scavenger receptor CD36. One of the main characteristics of senescent cells is secretion of SASP (such as GDF15, IL-6, IL-8, CXCL-1, CCL-16; CCL-26; CCL-7, CCL-1, CXCL-6, CXCL-5, CXCL-4, and CXCL-12).[105] SASP-mediated nuclear factor κB signaling was linked to the DNA damage response.[106,114] In addition, prolonged activation of the insulin-like growth factor (IGF) pathway, often observed in NASH livers, stimulation of IGF/IGF receptor network (IGF-binding proteins [IGFBPs], including IGFBP-2, -3, -4, -5, and -6 and their regulators, IGFBP-rP1 and -rP2) may contribute to senescent HSC phenotype. IL-22 was also implicated in driving senescent phenotype in HSCs via induction of STAT3-SOCS3-p53–dependent mechanism.[115]

Mice lacking key senescence regulators are more susceptible to liver fibrosis due to increased proliferation of aHSCs in response to chronic liver injury and excessive ECM accumulation.[74] Genetic labeling of GFAP-targeted HSCs revealed that although senescent HSCs downregulate collagen type I expression,[2] and that this effect is associated with suppression of ECM deposition, excessive accumulation of senescent HSCs can provide a detrimental inflammatory environment damaging to the surrounding cells and tissues.[103,115,116] Therefore, several studies have proposed ablation of senescent HSCs as a novel strategy for antifibrotic therapy. Senescent myofibroblasts can be cleared by Natural killer cells and liver-specific cells $\gamma\delta$ T via induction of interferon γ, Fas receptor, TRAIL, or tumor necrosis factor receptor 1-caspase8/caspase3/caspase9–dependent apoptosis.[8,34,50,117–119] Development of chimeric antigen receptor T therapy has become instrumental in targeting specific cell populations in vivo. Administration of chimeric antigen receptor T cells that target and ablate specifically senescent HSCs ameliorated development of NASH-induced liver fibrosis in mice.[120]

Inactivated HSCs. Regression of hepatic fibrosis was reported in patients treated for HBV and HCV and in experimental models in mice upon cessation of toxic liver fibrosis.[73,121] When the etiological cause of liver injury is removed, liver fibrosis may regress. Regression of liver fibrosis is characterized by gradual suppression of hepatic inflammation, disappearance of fibrogenic myofibroblasts, and resorption of fibrous scar by myeloid-derived collagenases. Besides the disappearance of myofibroblasts, another important component of liver fibrosis regression is the change in phenotype of macrophages.[2] ECM degradation critically depends on the influx of BM-derived myeloid cells that produce MMP2, MMP9, and other MMPs that cleave collagen type I fibers.[2] These macrophages do not possess inflammatory phenotypes,[122] but instead mediate transition of aHSCs into a senescence-like phenotype, facilitate their apoptosis, and engulf cellular debris (Figure 2).[8]

Using the Cre-LoxP–based genetic labeling of aHSC (using Col-a1(I)$^{\text{Cre-YFP}}$ and VIM-CreER) and single-cell polymerase chain reaction of HSCs from alcohol- and/or CCl_4-induced mouse liver fibrosis, it has been demonstrated that 50% of aHSC apoptose, while approximately 50% of aHSC escape apoptosis after cessation of liver injury, inactivate, and acquire a phenotype similar to qHSCs during the regression of liver fibrosis.[41,56,73,74] Inactivated HSCs (iHSCs) are first detected 7 days after cessation of CCl_4 injury but constituted $\sim$50% of total HSCs 1 month after the onset of fibrosis resolution. These myofibroblasts return to the space of Disse and revert to an inactivated phenotype, which is similar to, but distinct from, the quiescent state.[41,56] Compared with aHSCs, iHSCs downregulate expression of fibrogenic genes (including Col1a1 and α-SMA, TIMP1, LoxL2, Spp1) but upregulate the expression of some quiescence-associated genes like PPARγ and Bambi to the level that is similar to qHSCs.[41] However, some quiescence-associated genes such as GFAP, Adipor1, Adpf, and Dbp are not re-expressed in iHSCs, indicating the difference between qHSCs and iHSCs.[41] By comparing the global gene

expression in HSCs depending on their stage of activation, several genes that are differentially expressed in qHSCs, aHSCs, and iHSCs were identified and can be used to distinguish different HSCs.[123,124] Moreover, compared with original qHSCs, iHSCs are more responsive to fibrogenic stimuli and can contribute to recurring liver fibrosis more effectively,[41,124] suggesting that iHSCs may retain some biological memory of being activated. This observation suggests that chronic liver injury may cause repetitive activation and inactivation of HSCs increasing their susceptibility to fibrogenic stimuli (Figure 2).

Human aHSCs also have an ability to inactivate when adoptively transplanted into the livers of naïve immunodeficient mice (that do not provide fibrogenic environment). The mechanism of HSC inactivation is not well understood. Only a few transcription factors, PPARγ and GATA6 were implicated in driving HSC inactivation. Mice deficient of PPARγ or GATA6 in aHSCs exhibit a defect in suppression of fibrogenic genes, HSC inactivation, and fibrosis resolution.[89]

Nonconventional HSC Phenotypes.

Embryonic HSCs. In adult mice, aHSCs drive production and secretion of ECM proteins in chronically injured liver, mostly collagen type I, that forms fibrous scar and makes the liver fibrotic.[43] HSCs are activated during embryonic development (to a lesser extent than CCl_4-activated HSCs).[41] In contrast to adult aHSCs, eHSCs did not secrete ECM, and did not cause fibrosis of the fetal liver. Specifically, studies of naïve Col1a1-GFP mice have determined that eHSCs (E16–E21) upregulated collagen type I in real time. Unlike adult aHSCs, eHSCs (E16–E21) only moderately upregulate fibrogenic genes (Col1a1, Col1a2, Timp1, Tgf) but uniquely induce a unique set of genes, such as Sec3, Serpinec1, Apoh, Ahsg, Cyp2d26, Apof, Pzp, Emr1, Marco, Ear11, Igh-6, Coro1a, and Rac2.[41] In addition, eHSCs (E16–E21) shared expression of top common genes with iHSCs, such as Ccl4 chemokine, Clec7a (Dectin1, a member of the C-type lectin/C-type lectin-like domain CTL/CTLD superfamily), Bcl2a1b (BCL2 related protein A1), Ctss (Cathepsin S), and Lgals3 (Lectin/Laminin binding protein, a member of galectin family of carbohydrate binding proteins),[41] suggesting that eHSCs (E16–E21) can inactivate (Figure 3*A*).

Persisting embryonic HSCs. Cell fate mapping of eHSCs (from Cola1-Cre × Rosa-flox-Stop-YFP mice) revealed that the majority of eHSCs (∼75%) apoptose in the livers of neonatal mice, and by 2 months of age were replaced with newly generated qHSCs (with no history of collagen type I expression).[41] Meanwhile, 20%–25% of embryonic eHSCs persisted in the naïve 2 months old $Col1a2^{YFP}$ mice.[41,56] peHSCs escape apoptosis, downregulate fibrogenic genes by neonatal day 21, and upregulate classical quiescent genes, and exhibit the identical gene expression profile similar to that observed in adult qHSCs. peHSCs and qHSCs share expression of the top genes such as Hhip, Rtp4, Thyh1, Hspa1b, Adcy5, Aldh1a3, Sod3, BY718203, Itga2b, Oas2, Disp2, Raet1a, and others,[41] suggesting that eHSC activation can be reversible to the original quiescent phenotype. Paradoxically, functional studies of inactivation of adult aHSCs into iHSCs revealed that iHSCs can only in part restore their quiescent-like phenotype, while eHSCs persisting in the livers of adult mice (peHSCs) exhibited a phenotype, which is undistinguishable from the classical qHSCs.[41] Further studies are needed to compare the transitionally activated eHSCs and chronically activated adult aHSCs phenotypes, as these findings can identify a new pathway of HSC inactivation and provide new strategies for antifibrotic therapy. Resolving the mechanism of eHSC inactivation into peHSCs may provide key targets to revert aHSC into qHSCs in patients with NASH (Figure 3*A* and *B*).

Recovery-associated qHSCs. Based on cell fate mapping experiments, the appearance of $Col1a2\text{-}YFP^{+}$ iHSCs was observed in recovering livers as early as day 7 after CCl_4 cessation. At this time, the vast majority of HSCs remaining in the recovering liver (after immediate apoptosis of 50% of aHSCs after CCl_4 cessation) were represented by iHSCs.[41] In turn, 2 weeks into liver fibrosis resolution, the composition of HSC population changed, and consisted of ∼70% iHSCs and ∼20% of HSCs without history of Col1a2-YFP expression (recovery-associated qHSCs [rqHSCs]). rqHSCs were newly produced in the liver and proliferated from an unknown source. 1 month after CCl_4 cessation, rqHSCs contributed already to ∼50%–55% qHSCs,[41] and the HSC population was replenished almost to the physiological numbers (6% vs 10% of total liver cells normal liver).[42] These results indicate that HSCs play a critical role during recovery from liver fibrosis, and their reconstitution may play a role in the maintenance of liver architecture and regeneration. Unexpectedly, rqHSCs shared more similarities in their gene expression profiles with iHSCs (such as Pex5l, Saa3, Wnt6, Gpnmb, Clic5, Rmrp) than with qHSCs, and uniquely upregulated Bmp10, Trim55, Cdk8, Crebzf, Pdpk1, and other genes that distinguished them from both iHSCs and aHSCs.[41] These findings outline the importance of the liver environment for HSC phenotypes. Although it remains unclear how the HSC niche is affected during fibrosis resolution, rpHSCs can acquire their unique phenotype due to close proximity to iHSCs or residual effect of stiff liver on HSC progenitors (Figure 3*A* and *B*).

Inflammatory HSCs and proliferative HSCs. scRNA sequencing is a groundbreaking technique that enables the sequencing of messenger RNA from tens of thousands of individual cells.[75,125] Bioinformatic analysis of these messenger RNA sequences uncovers intricate disparities in gene expression within tissues, resulting in a comprehensive understanding of the cellular landscape.[76] Recently, Rosenthal et al[126] examined the single-cell expression profiles of HSCs isolated from *foz/foz* mice (in which the Alms1 gene was mutated similar to that in patients with Alstrom syndrome), a murine model of NASH, which develop metabolic syndrome, insulin resistance, and NASH liver fibrosis after 12 weeks of feeding with Western diet.[127] scRNA sequencing analysis confirmed the presence of qHSCs, aHSCs, and iHSCs in these mice. Livers of control mice were enriched in qHSCs (comprising 2 defined subsets expressing periportal [Ngfr and Itgb3] or pericentral [Adamtsl2 and Rspo3] markers, while aHSCs from NASH-injured livers upregulated fibrogenic genes [Col1a1 and Acta2]). iHSCs were detected in mice recovering from NASH liver fibrosis (Figure 3*A* and *B*).[126]

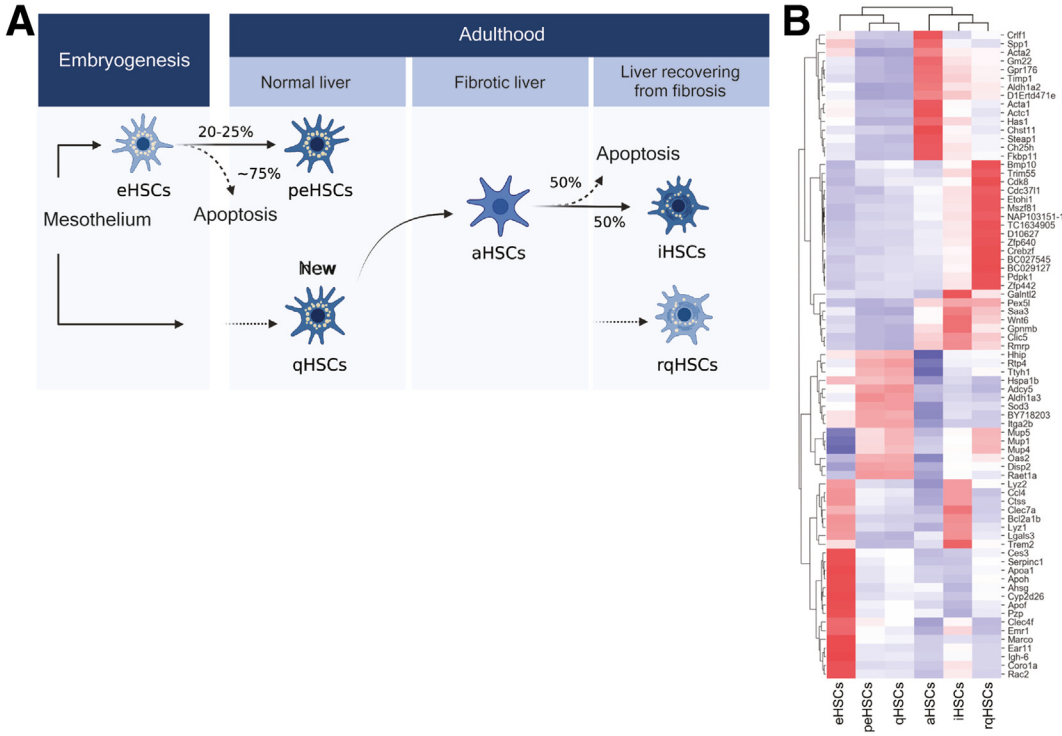

Figure 3. Potential similarities and differences in responses of embryonic and adult HSCs to injury and inactivation. (*A*) Based on the data analysis of Col-GFP mice (that express collagen type I in real time) and cell fate mapping of collagen type I–expressing HSCs throughout the embryonic development (E14–E16) into the adulthood (2 months), dynamic changes in the HSC composition in normal liver, in response to fibrogenic injury (2 months of CCl_4), and during regression of liver fibrosis (1 month after CCl_4 cessation), were identified. (*B*) The heatmap depicts the top genes uniquely expressed in eHSCs, eHSCs that escape apoptosis and persist into the adulthood in healthy mouse livers (peHSCs), the classical qHSCs, activated fibrogenic myofibroblasts (aHSCs), and HSCs detected during fibrosis resolution (HSCs that survive apoptosis and inactivate [iHSCs] and newly generated recovery-associated HSCs [rqHSCs]). Upregulated (red) and downregulated (blue) genes are shown.

In addition, scRNA sequencing identified 2 novel HSC clusters: inflammatory (INF) HSCs and proliferative HSCs. The INF HSC cluster expressed low levels of fibrogenic genes and highly upregulated inflammatory genes (Pdgfrb, CD36, Ly6C, Mrc1, CLEC, Fabp4, Selenop, Maf, Cavin2, Kdr, Mrc1, Aqp1, Ptgprb, and others), and their phenotype was regulated via activation of Foxc2, Sox7, Crem, Klf2/4, Tcf12, Gata2, Hoxa1/5, Bach1, and Tal1 transcription factors.[126] A proliferative cluster was functionally linked to fibrogenesis and proliferation. Many proliferation markers, such as Cdk1, were exclusively expressed in this cluster. Proliferative HSCs also uniquely upregulated Serping1, Irf7, Mustn1, Rgs5, Colec11, Plvap, Sod3, and Clca3a1 and were regulated by E2f7 and E2F3, Nrf1, and Nfya transcription factors. The proliferative cluster was mostly present in aHSCs but minimally induced in qHSCs and iHSCs. In turn, INF HSCs were present in qHSCs, aHSCs, and iHSCs.[126] Meanwhile, INF HSCs and proliferative HSCs were strongly associated with highly fibrogenic ECM-producing aHSCs and contributed to the activated regulon of NASH-activated HSCs. Although the exact role of INF and proliferative HSCs is not well characterized, most likely they play an important role in supporting collagen type I–producing HSCs (Figure 3*A* and *B*).[126,128]

Portal Fibroblasts

In normal liver, PFs are a small population of periductular mesenchymal cells that support the integrity of the portal tract.[27,60] Under physiological conditions, PFs contribute to ~0.1% of total liver cells. Therefore, purification of quiescent PFs from normal mouse livers is technically difficult, and the majority of studies were focused on the characterization of aPFs. In response to cholestatic liver injury, aPFs proliferate and rapidly upregulate production of ECM proteins (Figure 1).[61,129,130] aPFs do not migrate into the liver parenchyma, but rather are located in the portal tract, at the site where they originated, and produce fibrous scar. Activation of aPFs precedes activation of HSCs in experimental cholestasis.[55] Cholestasis-activated aHSCs exhibit more resemblance with aPFs than CCl_4-activated aHSCs. aPFs can be distinguished from aHSCs by expression of ectonucleotidase 2 (NTPDase 2), Thy-1,[51–53] fibulin 2,[51] elastin,[54] Msln, Muc16, Aspn, Bnc1, Upk1β, Calca, and Gpc3.[42,55] However, there is no single marker to define aPFs, and their heterogeneity poses a challenge to distinguish them in the liver.[131]

Msln, Thy1, and Muc16 Expression in aPFs. Thy-1 (CD90), originally discovered as a thymocyte antigen, is a GPI-linked protein, which is expressed in fibroblasts, T cells,

and neurons.[132] Thy-1 suppresses TGF-β–mediated PF activation. Thy-1 knockout mice are more susceptible to cholestatic fibrosis.[133] MSLN, another membrane-anchored, GPI-linked protein, was originally identified as a tumor marker expressed by solid tumors such as mesotheliomas and ovarian cancer, and serves as a target for anticancer therapy.[134,135] Msln is expressed in aPFs and promotes cholestatic fibrosis by assisting PF activation. MSLN is upregulated in mesothelial cells during embryonic development but minimally expressed in adult normal tissues[136] and therefore can potentially be used for targeting aPFs. Muc16, the murine counterpart of ovarian cancer antigen CA125,[137,138] was identified as the only known ligand of Msln. CA125 belongs to the family of mucins that are anchored to the cell membrane. In aPFs of cholestatic injury, MUC16 binds to MSLN and Thy-1 and transmits intracellular signaling from both GFP-linked proteins.[55]

Msln-Muc16-Thy-1 Signaling in aPFs. Msln expression in tissue fibroblasts could be connected to the molecular mechanism of PF activation through the profibrogenic pathway induced by TGF-β1.[55,139] Lineage tracing studies[42] have shown that Msln-positive aPFs contribute to liver fibrosis in mice with cholestatic fibrosis. Genetic ablation of Msln-positive aPFs successfully inhibited the progression of BDL-induced cholestatic fibrosis. Genetic deletion of aPFs ($Msln^{-/-}$ mice) attenuated development of BDL-induced fibrosis and cholestatic fibrosis.[55]

In additional to conventional TGF-β1-TGFβRI-Smad2/3/4 signaling, aPFs also induce Msln-Muc16-Thy-1 signaling pathway. Msln was shown to bind to Muc16 and Thy-1, regulating TGF- β1 signaling pathway in the activation of PFs.[55] The interaction between Thy-1, Msln, Muc16, and SMAD7 forms a complex that regulates the TGFβRI-TGFβRII complex. Msln and Muc16 enhance the TGF-β1 responses in aPFs, while Thy-1 and SMAD7 suppress the TGF-β responses. More specifically, MSLN facilitates TGF-β1-inducible activation of PFs by disrupting the formation of the inhibitory Thy-1-TGFβRI complex.[55,139] In addition, deletion of Msln in aPFs inhibited fibroblast growth factor–induced proliferation. The proliferation of aPFs driven by fibroblast growth factor was significantly reduced in $Msln^{-/-}$ aPFs. The fibroblast growth factor receptor 1 complex did not identify Msln-Muc16 as binding partners, and deletion of Msln directly suppressed AKT phosphorylation, which regulates proliferation.[55]

Targeting aPFs for the Treatment of Liver Fibrosis. Due to its minimal expression in normal tissues, MSLN has the potential to become a target for the treatment of cholestatic fibrosis. This observation led to the strategy to treat cholestatic fibrosis using immunotoxin-based ablation of human aPFs. Treatment with MSLN-coupled immunotoxins (engineered by attachment of PE38 toxin to anti-human MSLN SS1 antibody)[140–142] successfully removed human $GFP^{+}MSLN^{+}$aPFs that were adoptively transplanted into BDL-injured immunodeficient mice, and attenuated development of cholestatic fibrosis in these mice. Targeting of human MSLN with immunotoxins might provide a new insight into treatment of cholestatic fibrosis.[133]

Conclusions and Future Perspectives

There are no effective therapies of liver fibrosis of different etiologies. Many single-target therapies failed, perhaps reflecting the complexity of chronic liver injury. An emerging strategy for treatment of liver fibrosis is to halt fibrosis progression, and suppress fibrogenic machinery and production of fibrosis scar by hepatic myofibroblasts, while the underlying cause of chronic liver injury can be treated. Therefore, hepatic myofibroblasts are the primary targets for antifibrotic therapy.

aHSCs and aPFs were identified as primary sources of hepatic myofibroblasts in fibrotic liver. Older studies of cultured cells to determine their fate have produced artifacts, in which many cell types can become myofibroblasts on plastic but not in vivo. Extensive cell fate mapping in mice has demonstrated that HSCs are the primary source of myofibroblasts in hepatotoxic liver fibrosis. Recently, there has been renewed interest in portal fibroblasts as a source of myofibroblasts, particularly in cholestatic liver fibrosis.[143] Although there is clear heterogeneity, the overwhelming majority of the data support 2 cell types as the major sources of myofibroblasts: PFs and HSCs.[20,42] A role for epithelial-mesenchymal transition for mesothelial cells of the hepatic capsule to become myofibroblasts for capsular fibrosis is also possible. Vascular smooth muscle cells can exhibit contractile filaments and increase ECM synthesis in response to injury.[144] While this may not be their primary function, vascular smooth muscle cells expressed COL1A1 and COL3A1 in the liver fibrotic niche.[20] The physiology of aHSCs has been extensively studied, mostly due to their abundancy and persistence in the liver compared with aPFs. The population of aHSCs is heterogeneous, and distinct cellular subsets may contribute differently to the development of liver fibrosis.

Mesenchymal cells derived from the septum transversum that formed the mesothelium migrate into the liver to form both HSCs and portal fibroblasts.[77,145] So, there is expected overlap in phenotypes and markers between mesothelial cells, HSCs, and portal fibroblasts. Also, cellular markers change when quiescent cells are activated by a fibrogenic stimulus. Currently, researchers are reconciling cell fate mapping in mouse livers with scRNA sequencing and snRNA sequencing in mouse livers and human livers.

PFs have only been characterized and purified in their activated state. Recently, mesenchymal cells from the portal tract have been characterized using scRNA sequencing.[143] These cells are surprisingly heterogeneous. They include a portal mesenchymal stem cell (PMSC), which could be a source of aPFs. Like other MSCs, these cells do not fulfill the definition of a stem cell and should more appropriately be called mesenchymal stromal cells. Slit guidance ligand 2 (SLIT2) was identified as a prototypical gene of the PMSCs. Within fibrotic septa, $SLIT2^{+}/\alpha\text{-SMA}^{+}$ PMSC–derived myofibroblasts were intermingled with and outnumbered by $SLIT2^{-}/\alpha\text{-SMA}^{+}$ myofibroblasts and were frequently located in the surrounding of vascular lumens.[146] $Msln^{+}$ aPFs are proposed to align with the $SLIT2^{+}$ PMSC–derived myofibroblasts. Based on mouse gene expression datasets

after BDL, $GFP^{+}Vit.A^{-}$ myofibroblasts, which were identified as aPFs, highly express both Msln (66-fold compared with $GFP^{+}Vit.A^{+}$ myofibroblasts) and Slit2 (4.5-fold compared with $GFP^{+}Vit.A^{+}$ myofibroblasts).[41] Unlike HSCs, cell fate mapping has not been performed to determine the relationship between portal mesenchymal cells and aPFs. However, aPFs are clearly distinct from aHSCs and the role of MSLN in aPFs needs further study.[147]

In recent studies, single-cell/single-nucleus analysis of both human and mouse livers have demonstrated heterogeneity of HSCs.[125,148] In a study by Ramachandran et al,[20] hepatic nonparenchymal cells were isolated from healthy and fibrotic human livers and subjected to scRNA sequencing to generate an atlas of liver-resident cells. In the clustering of human liver mesenchymal cells, scar-associated mesenchymal cells (SAMes) were defined as the mesenchymal cell subpopulation that express PDGFRα and expand in cirrhotic livers. Based on the pseudotemporal ordering and RNA velocity analyses, the study demonstrated a trajectory from human HSCs to SAMes, which indicates HSCs differentiate to scar-producing myofibroblasts. Interactome modeling of ligand-receptor pairs between the nonparenchymal cell subpopulations suggested that scar-associated macrophages regulate SAMes activation and expansion in the fibrotic niche.[125] In a recent study, Wang et al[149] conducted snRNA sequencing to analyze NASH livers from both humans and mice. Their findings revealed the emergence of a profibrotic autocrine signaling loop in hepatic stellate cells during the late stages of the disease, consisting of several receptor-ligand pairs. snRNA sequencing data from livers of NASH patients and FAT-NASH model mice identified a conserved autocrine signaling circuit that consists of 68 ligand-receptor pairs.[149]

Future studies, specifically based on the single-cell technologies and spatial transcriptomics with specific focus on aHSCs, will provide a new insight into translation of experimental studies. Similar studies are urgently needed to characterize the subpopulations and fate mapping of mouse and human aPFs. Many emerging studies will aim to characterization specific cellular subsets, rather than the entire liver populations, and provide a deeper mechanistic characterization of the signaling pathways regulating activation of fibrogenic myofibroblasts in fibrotic liver.

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